CELL DIVISION IN PINE SEEDLINGS.[1]

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(WITH PLATES XXIII AND XXIV)

THE chief purpose of the following investigation was to determine the origin of the achromatic spindle, especially as to whether it originates as a bipolar or a multipolar structure. Along with this the subject of centrospheres was considered and also such other points of interest as might be observed in connection with karyokinesis.

The greater part of the work was done on *Pinus laricio* Poir., *P. silvestris* L. being used for comparison. The cell structures in the two species were found to be so nearly identical that it was not considered necessary to make any distinction between them in presenting the results of the investigation.

The material was obtained by sprouting the dry seeds, and when the embryos were from 0.5–3^{cm} long the root tips and cotyledons were cut off and killed in the usual manner. The fixing agents used were Flemming's stronger solution and chrom-acetic acid. The sections were imbedded in paraffin, and cut 10, 5, and 4 μ thick. Various combinations of stains were used; but the best results were obtained with analin-safranin gentian-violet, and orange G; iron-alum-hæmatoxylin; and Delafield's hæmatoxylin. The sections were usually stained so dark when killed in Flemming's solution that it was necessary to let them stand in turpentine exposed to the sunlight for some time in order to remove the black color which otherwise interfered greatly with the proper effect of the staining reagents. After this treatment the sections stained very well, and the details of protoplasmic structure were well differentiated.

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[1] Contribution from the botanical laboratory of Ohio State University. IV.

The cells of Pinus are moderately large and the karyokinetic figures distinct, but usually there is a considerable amount of oil present which is stained readily by most of the stains used, and thus interferes with the observation of the finer details of structure, especially with the centrospheres.

In the resting nucleus (*fig.* 1) the chromatin is arranged in a granular network with rather large meshes. Several nucleoli of various sizes are usually present, surrounded by hyaline areas. In normal cells of this stage no centrospheres were observed, although a diligent search was made for them. This might be taken to indicate that centrospheres are entirely absent in the resting stage, which would be contrary to the condition Schaffner[2] reported for *Allium Cepa*, where centrospheres are said to occur in resting cells as well as in the stages of division. However, the cytoplasm usually contained many oil drops and other granular contents so that if centrospheres were present they could not be distinguished very easily. In the outer layer, near the epidermis, elongated cells are sometimes found (*fig.* 3) which contain greatly elongated nuclei having spindle-like projections with bodies resembling centrosomes at the outer ends. Sometimes these bodies have radiations around them which make them strongly resemble the poles of a true spindle. Although the nucleus is in the resting stage the bodies might represent the poles of spindles which formed earlier than usual. However, the cells in this region seldom divide, and the phenomenon may be only an accompaniment of the elongation of the nucleus, the centrosome-like bodies representing accumulations of cytoplasm.

In the stele the elongated cells (*fig.* 4) contain very large nuclei having numerous nucleoli. In such cases the nucleoli are usually very large and filled with vacuoles. The nucleoli stain very dimly, so that the chromatin network is hardly visible even in well stained sections, while the nucleoli take a deep red stain with safranin, and are arranged in a line within the nucleus at somewhat regular intervals.

[2] BOT. GAZ. **19**: 445–459. 1894.

In some of the preparations very definite and distinct radiations surrounded the nuclei (*fig. 2*). These radiations are rather thick strands of cytoplasm, and do not show the fine structure found in spindle threads or in radiations around the poles. In the preparations where such radiations were seen nearly every cell showed them, whether in the resting condition or the early stages of division; indicating that they were produced by the fixing agent. If not caused by this means they are probably the result of a streaming of the cytoplasm similar to that found in the cells of hairs on Tradescantia stamens.

Just before cell division commences the nuclei stain very readily. The first change to take place in the structure of the nucleus is the transformation of the chromatin network into a long thread or spirem. When the spirem is almost formed, and while the nucleoli are still visible in the nucleus, and before the nuclear membrane has disappeared, the spindle begins to form. When first seen it consists simply of two rounded or dome-shaped prominences (*fig. 5*), one on each side of the nucleus (*figs. 6–10*). These dome-shaped spindles gradually become elongated and pointed until they extend outward to two definite points at which centrosomes are often visible (*figs. 10–12*). These appear as small deeply stained bodies placed just at the point of the spindle (*figs. 24, 25*). In *fig. 16* the centrosome appears to be lying in a hyaline area which is surrounded by a darker portion of protoplasm. At about the time the spindle becomes pointed the spirem breaks into a definite number of chromosomes and the nuclear membrane disappears. At this time the nucleoli are no longer visible, having disappeared in the early prophase of division. They are not found again until cell division is about complete. The poles are usually approximately on opposite sides of the nucleus from the first appearance of the spindle. In a few cases, however, they were less than 180° apart (*fig. 12*),.and did not become directly opposite until quite a late stage of karyokinesis. Radiations are frequently seen around the poles (*figs. 6, 11*).

F. Rosen[3] describes spindles quite similar to the above in the root tips of *Hyacinthus orientalis*. He finds the spindle arising as a cap-shaped prominence which is formed on two opposite points of the nucleus by the concentration of "kinoplasm" which had formed a hyaline area around the nucleus. This spindle originates before the dissolution of the nuclear membrane.

I examined carefully a large amount of material in search for radiations around the nuclei and for multipolar spindles in the prophases of karyokinesis as figured by Osterhout,[4] Mottier,[5] Juel,[6] and Debski,[7] but I was unable to find a single cell in these early stages that showed such structures. However, as above observed, in some preparations strands of cytoplasm were seen around the nuclei of nearly every cell. This was observed in cells both in the resting stage and during karyokinesis. These radiations were very coarse and could not, I think, be instrumental in spindle formation.

In some injured or sliced karyokinetic figures I found, though very rarely, spindles which appeared to be multipolar. The cells containing such spindles were all in the anaphase, mainly in the metakinesis and mother star stages (*fig. 21*), at which time the spindle is elongated and is more likely to be sliced in sectioning than those which are in other stages of karyokinesis.

In the material examined many cells were observed in the prophase, a large number of which showed definite spindles. In every case the spindle was bipolar, being short and rounded, or dome-shaped, when first visible near the nuclear membrane. The evidence furnished by my investigation is opposed to the theory that the spindle of Pinus originates as a multipolar structure.

With the segmenting of the spirem the metaphase begins. The spirem is scattered throughout the nucleus in the outer

[3] COHN'S Beiträge zur Biologie der Pflanzen **7**: 225–312. 1895.

[4] Jahrbücher fur wiss. Bot. **30**: 159–168. 1897.

[5] *Ibid.* **30**: 169–204. 1897.

[6] *Ibid.* **30**: 205–226. 1897.

[7] *Ibid.* **30**: 227–248. 1897.

part of the achromatin (*figs. 9–11*), and segments just before the spindle elongates to definite points. The nuclei in which I was able to count the segments contained sixteen chromosomes (*fig. 14*). They are somewhat difficult to count as they are usually massed together. Strasburger[8] counted twelve chromosomes in the pollen grain of *Pinus silvestris*. Dixon[9] found eight, twelve, and twenty-four chromosomes in the gametophyte of *Pinus silvestris*, with eight as the prevailing number. The nuclei in the primary meristem of the growing point of *Pinus Laricio* and *Picea orientalis* were found by the same author to contain sixteen chromosomes.

The chromosomes are at first scattered throughout the nucleus, but are gradually drawn toward the center to form the mother star (*fig. 15*). They seem to be arranged somewhat irregularly during this and the metakinesis stages (*figs. 15, 20, 22*). The longitudinal splitting of the chromosomes takes place about the time of the mother star stage. The daughter chromosomes then move toward the poles where they arrange themselves into the two daughter stars and form the network of the resting nuclei (*figs. 28, 29*). The spindle is usually quite pointed during metakinesis (*fig. 22*). Sometimes, however, when the cell is short, the spindle does not become pointed but remains dome-shaped (*fig. 18*), giving an appearance similar to that which would be produced by the spindle fibers passing through the cell wall. The centrosomes, however, show that the poles lie very close to the cell wall. *Fig. 17* shows one end of such a spindle with a double centrosome. In *fig. 24* the sides of the spindle are concave. This shape was probably produced by the protoplasm contracting near the lower end of the spindle. Radiations are more prominent during the anaphase than during either the earlier or later stages (*figs. 19, 23, 25*).

The centrosomes in Pinus appear as small but definite and readily stained bodies lying at the poles. Sometimes a hyaline area is visible around them in which the spindle threads termi-

[8] Ueber das Verhalten d. Pollens. Hist. Beitr. **4**: —. 1892.

[9] Ann. Bot. **8**: 21–34. 1894.

nate. In other cases no such area can be observed, the spindle fibers appearing to meet at the centrosome. These bodies, on account of their common occurrence at the poles, should perhaps retain the name of centrosomes whether they are permanent bodies directing cell division or whether they are only temporary structures. Whatever these bodies may be they certainly seem to be the same in character as the bodies found at the poles of cells in animal tissue. They are not only the points to which spindle fibers converge, but they are also the centers for a system of radiations which pass outward into the cytoplasm.

H. L. Smith[10] is perhaps the first to have figured centrosomes in plants. He found a small body in diatoms, especially in *Surirella splendens*, which he called the germinal dot. This was no doubt a centrosome. Guignard[11] figured and described these bodies in resting cells as well as during karyokinesis. In his recent paper[12] he finds centrosomes in all phases of nuclear division in *Nymphæa alba*. He finds multipolar spindles also in *Nuphar luteum*, and says they are very frequent in *Limodorum abortivus*, but he does not give any explanation of their origin.

In the early part of the telophase the cell wall between the two daughter nuclei begins to form. It starts as a granular thickening of the middle of the central spindle fibers (*fig. 26*). This thickening gradually extends outward and the spindle at the same time gradually increases in diameter (*figs. 27, 28*) until its middle portion touches the cell walls. The cell plate then completely divides the daughter cells and the spindle soon disappears. In *fig. 28* traces of it may still be seen. Two centrosomes are now found at each pole, the single ones having divided. While the network is being formed the daughter nuclei change from an oval to a spherical form, sometimes hav ing radiations around them (*fig. 29*).

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[10] A contribution to the life history of Diatomaceæ, Proceedings American Society of Microscopists **1886**: 1–37.

[11] Ann. Sci. Nat. (Bot.) VII. **14**: 163–296. 1891.

[12] BOT. GAZ. **25**: 158–164. 1898.

EXPLANATION OF PLATES XXIII, XXIV.

The figures were drawn with combinations of Zeiss and Bausch and Lomb objectives and oculars by the aid of a camera lucida, and are reduced to about $\frac{3}{8}$ of their original size. The initial letter of the objectives and oculars are used to designate them. The four following combinations were employed: Z 2^{mm} ap. Z 18 (× 2250); Z 2^{mm} ap. Z 12 (× 1500); B & L $\frac{1}{12}$ Z 12 (× 2600); B & L $\frac{1}{12}$, B & L $\frac{3}{4}$ (× 1400).

Figures 1, 16, 17, 18, 26, and 29 were taken from *Pinus silvestris;* all the others from *Pinus Laricio*.

PLATE XXIII.

FIG. 1. Resting nucleus with large oil drops in the cytoplasm. B & L $\frac{1}{12}$ Z 12.

FIG. 2. Resting cell, with radiating streams of cytoplasm around the nucleus. B & L $\frac{1}{12}$; B & L $\frac{3}{4}$.

FIG. 3. Resting cell containing an elongated nucleus with a spindle and centrosome-like bodies. Z 2^{mm} ap. Z 18.

FIG. 4. Part of cell containing a very large nucleus in which are several nucleoli with vacuoles. B & L $\frac{1}{12}$ Z 12.

FIG. 5. Early stage of division showing first appearance of spindle. B & L $\frac{1}{12}$ Z 12.

FIG. 6. Spindle elongating showing centrosomes and radiations. B & L $\frac{1}{12}$ Z 12.

FIG. 7. Same stage, somewhat later, showing few radiations. B & L $\frac{1}{12}$ Z 12.

FIG. 8. Dome-shaped spindle showing neither centrosomes nor radiations. B & L $\frac{1}{12}$ Z 12.

FIG. 9. Dome-shaped spindle showing centrosomes and a few radiations. B & L $\frac{1}{12}$ Z 12.

FIG. 10. Same stage more advanced. Z 2^{mm} ap. Z 12.

FIG. 11. Spindle becoming pointed showing centrosomes and radiations. Z 2^{mm} ap. Z 18.

FIG. 12. Spindle pointed but poles not entirely opposite. B & L $\frac{1}{12}$ Z 12.

FIG. 13. Nuclear membrane absent; spindle not so much pointed as is usual at this stage. B & L $\frac{1}{12}$ Z 12.

FIG. 14. Loose mother skein stage showing appearance and number of chromosomes. Z 2^{mm} ap. Z 18.

PLATE XXVI.

FIG. 15. Mother star stage. B & L $\frac{1}{12}$ Z 12.

FIG. 16. Showing one end of spindle with a large centrosome; chromosomes massed together. Z 2^{mm} ap. Z 12.

FIG. 17. Same as above with pole of spindle near cell wall. Z 2^{mm} ap. Z 12.

FIG. 18. Metakinesis; poles of spindle crowded against cell walls. Z 2^{mm} ap. Z 12.

FIG. 19. Metakinesis; pole with centrosome and distinct radiations. B & L $\frac{1}{12}$ Z 12.

FIG. 20. Metakinesis. Z 2^{mm} ap. Z 12.

FIG. 21. Metakinesis; injured, showing multipolar spindle; chromosomes are partly displaced. B & L $\frac{1}{12}$ Z 12.

FIG. 22. Metakinesis. B & L $\frac{1}{12}$ Z 12.

FIG. 23. Near close of metakinesis, showing centrosomes and radiations. Z 2^{mm} ap. Z 12.

FIG. 24. Same as above. Z 2^{mm} ap. Z 12.

FIG. 25. Daughter star with centrosomes and radiations. B & L $\frac{1}{12}$ Z 12.

FIG. 26. Loose daughter skein. B & L $\frac{1}{12}$ Z 12.

FIG. 27. Close daughter skein; cell plate formed. Z 2^{mm} ap. Z 18.

FIG. 28. The same but somewhat more advanced. Z 2^{mm} ap. Z 18.

FIG. 29. Near end of close daughter skein; nuclei are becoming globular. B & L $\frac{1}{12}$ Z 12.